# Evaluation of Breast Cancer Migration in Hydrogel Models

UNDERGRADUATE HONORS RESEARCH THESIS

Presented in Partial Fulfillment of the Requirements for the Bachelor of Science with Honors Research Distinction in the College of Engineering of The Ohio State University

By

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## Abstract

Each year, it is expected that over 250,000 women in the United States will be diagnosed with breast cancer and over 40,000 women will die from the disease[1]. In some cases, breast cancer cells metastasize and form secondary tumors in other organs, such as the liver, the lungs, bones, and the brain[2], causing a significant decline in survival rates[3]. It is not well understood why breast cancer cells behave differently from organ to organ. The goal of this study is to develop an understanding of breast cancer behavior in the brain environment by studying breast cancer cell lines in models mimicking the breast and brain tissue. In this study, 2 breast cancer cell lines and one normal mammary cell line were studied either on the surface of or encapsulated in collagen-hyaluronic acid (HA) hydrogel composites. The breast cancer cell lines MDA-MB-231 and MCF7 were under investigation, along with MCF10a, a healthy breast epithelial cell line, as a control. Collagen-I/III and HA were chosen because of their respective abundance in the breast and brain[4,5]. Migration and morphology patterns were analyzed to characterize differences between cell lines and hydrogel compositions. Results of this study revealed that migration of MDA-MB-231 cells was not hindered by the addition of HA, whereas migration of MCF-7 cells declined significantly with the addition of HA. This study reveals that these hydrogel models can provide viable methods to further investigate the migration patterns of invasive cancer cell cultures.

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# Chapter 1. Introduction

## Background

The search for treatments and therapies for those afflicted with breast cancer is an ongoing process. In order to develop the highest quality treatment, the means of migration and growth of breast cancer must be better understood. In an ideal setting, all cancer could be biologically characterized, resulting in an abundance of treatment options, thus reducing the lethality and symptomatic issues of cancer. Currently, women diagnosed with breast cancer in its early stages have a 100% chance of survival for 5 years after diagnosis, whereas women diagnosed with late stage breast cancer have a 22% chance of survival within the same time frame[2]. As a result of deficiency in knowledge about breast cancer metastasis and post-metastatic disease, modern medicine lacks the ability to characterize and predict tumorigenic cell behavior in differing stages of cancer development. By further identifying key characteristics of breast tumorigenic cells, this study aims to advance the understanding of breast cancers, specifically those in stage IV.

The progression of cancer development within the body is typically categorized into five stages of cancer, with the extent of cancer growth increasing with each step. In stage zero, no cancer has developed, only abnormal cells are present within a small area of the body. In stage I, cancer has developed within a small area in the body. In stages II and III,

the cancer has grown larger into a tumor and can grow into nearby tissue and lymph nodes. At stage IV, the cancer has travelled through the cardiovascular system to spread and grow within other areas of the body. At this point, the cancer can be considered advanced or metastatic[6]. For cancer cells to spread throughout the body, a series of migratory steps are required. First, the cancer cells must be capable of growing into or invading nearby normal tissue. Then, the cells must be capable of moving through the endothelial walls of nearby lymph nodes or blood vessels. Once this is possible, the cancer can circulate throughout the body within the circulatory system. The cells can then stop in small blood vessels or capillaries, and move through the blood vessel walls. The cancer then invades that nearby tissue and grows[7]. Once the cancer has grown into a tumor in this secondary position (hence why this tumor is called a 'secondary tumor'), the tumor then stimulates the growth of new blood vessels from existing vasculature to feed the expanding tumor[8].

Once the cancer is capable of metastasis, treatments are rarely curative. In previous years, a large center of focus was around treating the primary tumor in the originating area. Improving the understanding of metastatic cancer biology could prove key to developing better therapies to address metastasized cancer that might be resistant to standard treatments because of possible changes at the genetic and epigenetic level[9].

The most frequent sites for secondary growth resulting from metastasized breast cancer include the liver, lungs, bones, and brain[2]. Once the cancer has spread to these distant sites, the 5-year survival rate drops to 27%[10]. Patients diagnosed with metastatic breast cancer within the brain tend to receive the worst prognosis in terms of patient life outlook, with a median survival ranging from 2 to 25.3 months, along with a greatly reduced quality of life[11]. For the most invasive category of breast cancer, triple negative breast cancer, the survival time is at its lowest, at 3 to 4 months for patients that have developed a secondary growth in the brain[11]. In comparison, HER2-positive breast cancers, the next leading breast cancer type for brain metastasis, show longer reported survival rates even with a higher rate of brain metastases[11]. This project will only focus on breast cancer metastasis in the brain.

A primary goal of this study will be to investigate the biological response of breast cancer cells when exposed to environments that resemble the breast and brain. By tracking cell behaviors within sophisticated extracellular matrix-mimicking models, more information concerning invasivity and treatment options can become more evident. Within this study, I will be incorporating the use of complex hydrogel solutions to encapsulate cells, and I will study their response to these breast and brain-mimicking three dimensional (3D) environments. Along with these models, I will be positioning cells from the same cancer cell

lines on hydrogel solution surfaces to observe their behavioral response on a flat two-dimensional (2D) surface compared to that of a fully 3D encapsulated behavior.

## Hydrogels

Hydrogels, or 'hydrophilic gels', are composed of three-dimensional networks of polymer chains within an aqueous dispersion medium. What separates hydrogels from traditional colloidal gels, which include solid particle networks within a liquid solvent, is that hydrogels are capable of retaining a large ratio of water in comparison to the crosslinking material[12]. The properties of hydrogels provide distinct advantages of increased biocompatibility, tunable biodegradability, and porous structures deriving from the nature of the polymer chain networks. Hydrogels are considered prime candidates for applications in biosensing, drug delivery, and as carriers or matrices for cells in tissue engineering[13]. In a previous study, gelatin methacrylamide (GelMA)-based hydrogels were synthesized to support *in vitro* and *in vivo* spheroid-based models to investigate ovarian cancer in its advanced stages[14].

Hydrogel 3D models are used because the behavior of 3D-cultured cells is more reflective of physiological cellular responses *in vivo*. Research has shown that cells studied in 3D environments exhibit morphologies and physiologies that better reflect *in vivo*conditions than

those cultured in 2D environments[15]. Important differences that will be critical to this study between 2D and 3D modeling include differentiation, gene expression, general cell function, morphology, and proliferation changes. The effects of cell morphology in 2D vs. 3D can be seen in Figures 1 and 2.

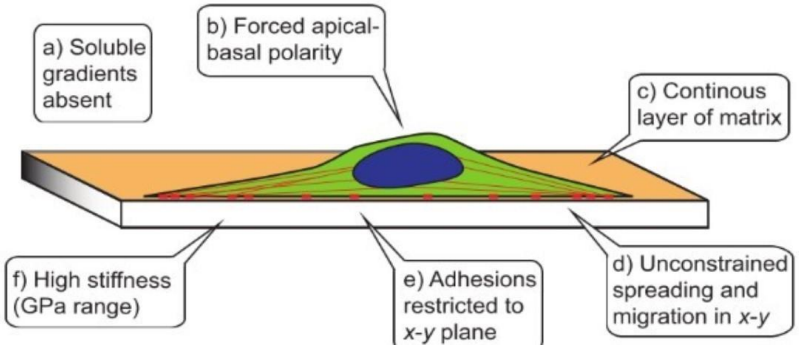


Figure 1: Characteristic behavior of cells grown on a 2D glass surface[15]

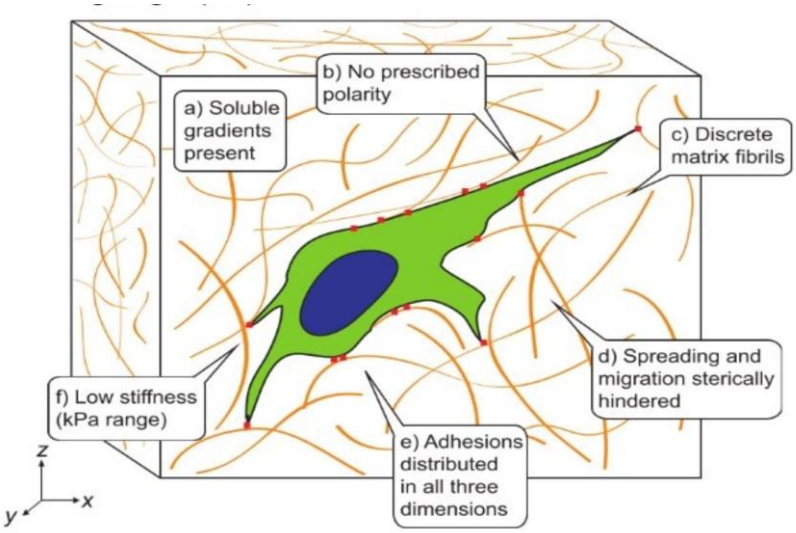


Figure 2: Characteristic behavior of cells grown within 3D collagen gel[15]

## Collagen

Collagen is the main structural protein of the extracellular matrix in various connective tissues in the body, including the breast. Collagen alone makes up 25% to 35% of the entire body content of mammals[16]. In this investigation, fibrillar type I collagen was used because it is considered the most abundant within the body, making up over 90% of the collagen within the human body[12]. Collagen hydrogels have also been widely used in 3D scaffolding because they more closely mimic *in vivo* anatomy than traditional cellular monolayers supported on tissue culture polystyrene[17]. For this investigation, collagen will be the primary building block for most hydrogel models, and hydrogels with polymer chain networks consisting only of collagen will be used to characterize cell behavior within the breast environment.

## Hyaluronic acid

Hyaluronic acid (HA) is an anionic, nonsulfated glycosaminoglycan distributed widely throughout connective, epithelial, and neural tissues[18]. Within the brain, HA is in high abundance along with lecticans and proteoglycans that contain a lectican and HA binding domain. The extracellular matrix within the brain is thought to provide resistance to invading cells of a non-neural origin[18]. For this reason, HA is used within this investigation to model the brain extracellular matrix in

order to study the cellular response of the breast cancer cells when exposed to this environment. It is also worth noting the presence of HA within extracellular matrices contributes significantly to cell proliferation and migration and may also be involved in the progression of some malignant tumors[19]. In cell migration, HA serves several functions ranging from purely structural to developmental regulation via control of tissue macro- and microenvironments[20].

HA is an important factor in cell migration because of its innate physicochemical properties and its direct interactions with cells. HA directly interacts with three principal cell receptors, CD44, RHAMM, and ICAM-1[21]. Although RHAMM is the receptor most attributed to cell migration[21], the cell receptor of most importance within this group is CD44, as it is a common marker of breast cancer stem cells[22]. A previous study showed that CD44 is a highly important cell receptor, as CD44 interactions with HA strongly stimulate migration in all tested glioma cell lines, inducing promoting cell detachment and invasion[23].

## Cell lines

For this investigation, three cell lines, MDA-MB-231, MCF-7, and MCF10a were individually studied in similar experimental models in order to find distinct differences in cellular response to hyaluronic acid and collagen within 2D and 3D culture environments. Each cell line

provides unique characteristics in terms of invasivity and proliferation, and these were used to provide more depth to the investigation.

MDA-MB-231

The MDA-MB-231 cell line is an epithelial, human breast cancer cell line that was established from a pleural effusion of a 51 year old, Caucasian female with a metastatic mammary adenocarcinoma. MDA-MB-231 is one of the most studied breast cancer cell lines in medical research laboratories. It is highly aggressive and highly invasive. This cell line lacks estrogen receptors and progesterone receptors ($ER,PR^-$), while amplifying human epidermal growth factor 2 ($HER2^+$) production[24]. Because of these mutations, MDA-MB-231 can be classified as a poorly differentiated triple-negative breast cancer, which also matches the aggressive nature of the cell line. MDA-MB-231 shows expression of the $CD44^+CD24^-$/low phenotype, which is similarly found in mammary cancer stem cells and characterizes cells with highly invasive properties[24].

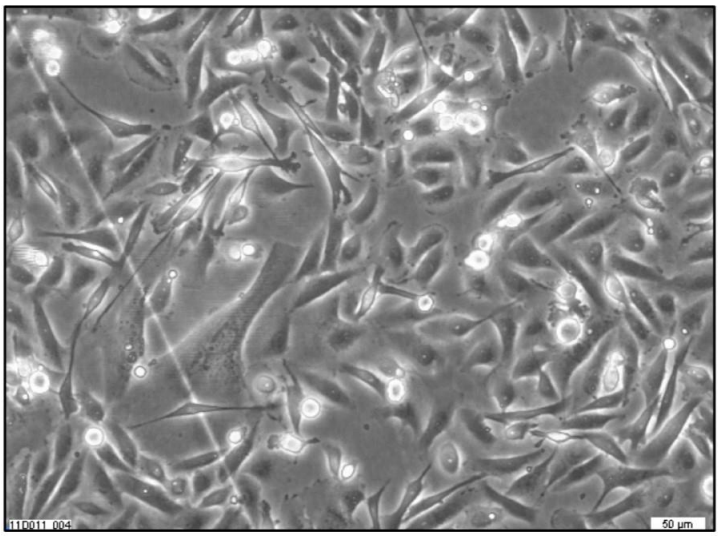

Figure 3: MDA-MB-231 cells in culture[24]

MCF7

MCF-7 is a breast cancer cell line isolated from a 69-year old, Caucasian woman in 1970[25]. The cell line for MCF-7 was established in 1973 and was the first mammary cell line capable of living longer than a few months. Being the first immortalized mammary cell line, as well as the first immortalized breast cancer cell line, to be applied within medical laboratories, MCF-7 has been widely investigated and understood through years of research[26]. MCF-7 is characterized as a primary tumor. MCF-7 does exhibit response to estrogen, and has been shown to be HER2 negative ($HER2^-$) and to exhibit estrogen and progesterone receptors($ER/PR^+$)[25].

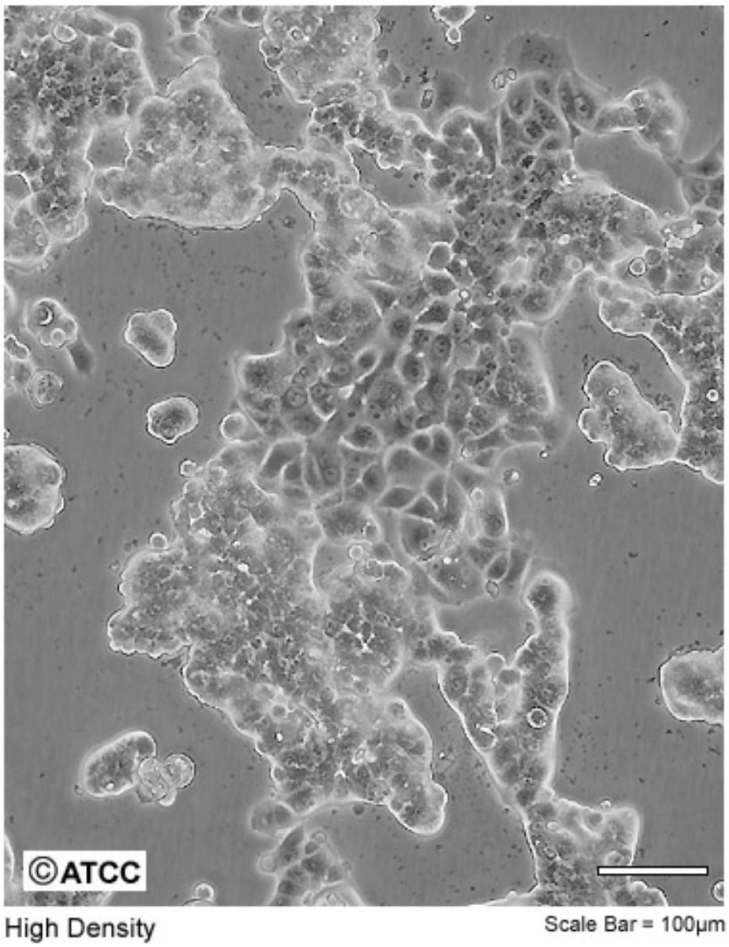


Figure 4: MCF7 cells in culture[27]

MCF10a

MCF10a is a human breast epithelial cell line that is likely the most applied normal breast cell line within medical laboratories[28]. The cells were derived from benign proliferative breast tissue. They are not considered tumorigenic, and their immortalization is mostly associated with depletion of the genes p16 and p14ARF, which regulate cellular

senescence[28]. For the purpose of this investigation, MCF10a is used as a control group to compare the behavior of the breast cancer cell lines MDA-MB-231 and MCF7 with the expected behavior of normal mammary cells.

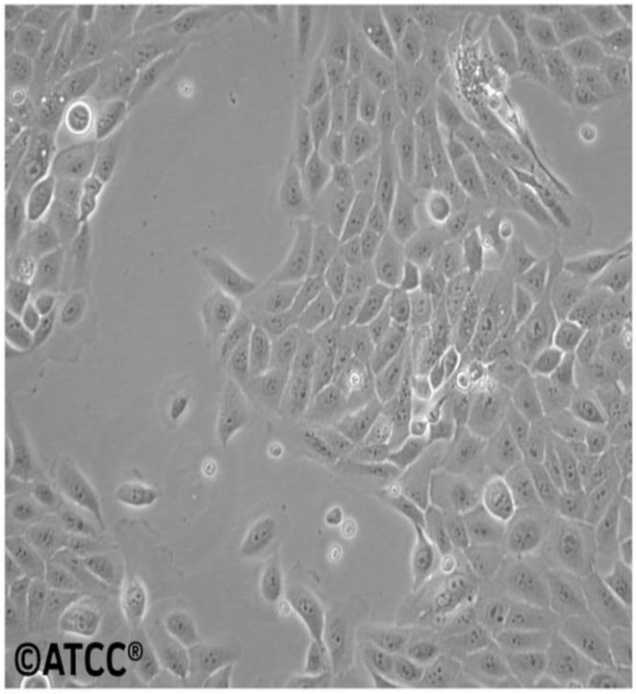


Figure 5: MCF10a cells in culture[29]

Cell behavior: Migration and morphology

The primary goal of the study is to investigate the behaviors of cell lines MDA-MB-231, MCF7, and MCF10a, within collagen, HA, and collagen-HA composite hydrogels. Tumor cells exhibit primarily two distinct modes of migration when invading the 3D environment: mesenchymal movement and amoeboid movement. These two types of

invasive movements are interchangeable due to cellular plasticity: suppression or enhancement of the activity of specific molecular pathways can cause a switch from one type to another. This change has been termed the mesenchymal amoeboid transition (MAT), or alternatively the amoeboid mesenchymal transition (AMT)[30]. MAT and AMT can occur quickly based on the specifics of the environment surrounding the cell in question. Each movement pattern has its unique advantages and disadvantages.

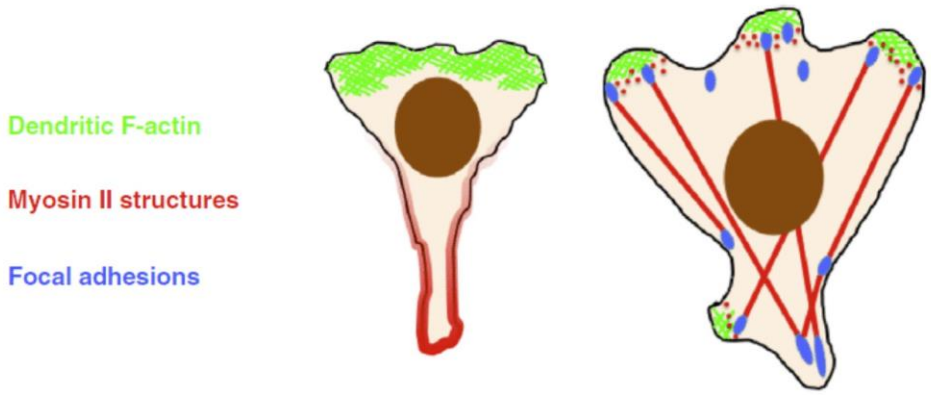


Figure 6: Visual representation of amoeboid (left) and mesenchymal (right) morphologies[31]

Mesenchymal movement

The mesenchymal type of migration is similar to the movement of fibroblasts. Cells with this type of motility have a specific, elongated,

spindle-like shape. In a 3D space, like the one created within this investigation, the cells polarize, showing an obvious leading edge with a lagging body[30]. Actin generates traction forces between the polarized edges, and clustered integrins provide focal adhesions that then recruit ECM-degrading proteolytical enzymes to remodel the nearby ECM and provide a path by which the cell can migrate[30]. This movement type is believed to be somewhat slower because of slow turnover of focal adhesions during translocation[30].

### Amoeboid movement

The amoeboid type of migration is named based on its likeness to a specific type of motility found in amoebas, characterized by cycles of expansion and contraction of the cell body mediated by localized actin and myosin[30]. Tumor cells that adopt an amoeboid-like invasive pattern have a characteristic round shape in 3D matrices. The low adhesion attachment style found within amoeboid movement allows cells to travel through 3D environments at relatively high speeds[30], barring physical barriers in the matrix.

## Expected results

The formation and use of 3D *in vitro* environments has been a topic of several studies that examine cancer cell behavior[32]. Evidence

shows that characteristics of tumor cell growth in 3D *in vitro* environments better reflect *in vivo* tumor cell growth behavior than those seen in 2D *in vitro* environments because 3D environments capture unique cell-cell and cell-matrix interactions[33]. Numerous breast cancer cell lines have been found to display different cell morphology and gene expression patterns across 2D and 3D environments[34]. Cancer cell invasion rates have been found to correlate with the generation and presence of HA within the surrounding extracellular matrix (ECM)[35]. The morphology of cells within 3D environments has also been correlated to levels of invasiveness based on morphologic factors[36,37].

Based on this, there are several hypotheses that were formed for this experiment. These results will be centralized around each cell line's migrational and morphological response resulting from the presence of HA within the hydrogel environment and from the placement of cell in 2D or 3D environments.

General trends

In an overall comparison of the three mammary cell lines, MDA-MB-231 is expected to migrate at a faster rate than MCF7, as MDA-MB-231 has been characterized as a more invasive cell line[24]. MCF10a is expected to migrate the least of the three because MCF10a is the control group that should resemble normal mammary cell behavior within this

study. Similarly, MDA-MB-231 is expected to exhibit more elongated, mesenchymal-like movement than MCF7. MDA-MB-231 is considered more capable of metastases because of its categorization as a poorly differentiated triple negative breast cancer as well as its distinct characteristics of invasion, due to their stellate projections that often bridge multiple cell colonies in 3D cell culture[24]. For this reason, MDA-MB-231 is expected to exhibit more elongated cell shapes that correlate with mesenchymal-like migration. Since MCF7 is categorized as less invasive than MDA-MB-231, this cell line is not expected to exhibit a morphology as elongated as MDA-MB-231. Since MCF10a is not expected to show any signs of migration, the morphology of the cells should be spheroid in nature and should show no signs indicative of elongation[28].

Response to HA

MDA-MB-231 is expected to have improved migration rates in comparison to the other cell lines when in the presence of HA in the hydrogels. MDA-MB-231 has a $CD44^{+}CD24^{-/}$low phenotype, thus increased expression of CD44 protein receptors should allow for increased adhesion and movement throughout a hydrogel containing HA polymers[24]. MCF7 is expected to not have improved migration rates in the presence of HA. Unlike MDA-MB-231, MCF7 does not overexpress

CD44 protein receptors, and as such, is less likely to adhere to HA polymers and migrate through an HA-containing hydrogel[24]. For this reason, MCF7 is expected to have decreased movement speed resulting from the lack of adherence to HA and because of increased movement resistance in the presence of the additional polymer chains of HA. MCF10a is not expected to show any major response in migration or morphology resulting from the presence of HA. Since MCF10a is the cell line that is used to mimic normal mammary cell behavior as a control within this experiment, no migrational response is expected with or without the inclusion of HA[24].

Differences between 2D and 3D conditions

The general expected trend across all cell lines is that cells will decrease migration speed when encapsulated in 3D hydrogel models compared to that observed when deposited on hydrogel surfaces in 2D models. The reasoning for this is that the increased resistance of polymer chains within the 3D model will add extra resistance to movement. It is expected that MDA-MB-231 could possibly experience less resistance between 2D to 3D models because of this cell line's ability to elongate and adopt a more mesenchymal-like migration behavior[24]. The reasoning for this is that MDA-MB-231 cells will be able to squeeze through spaces between polymer chains within the hydrogel more readily because of

their ability to elongate. Previous studies have found that MDA-MB-231 are able to readily squeeze through junctions, adding to their characterization as a more aggressive cell line[24]. Since MCF7 is expected to migrate in a more amoeboid-like fashion, it will have a more difficult time migrating through a 3D hydrogel likely because of increased resistance from the polymer chains. MCF10a will likely not show any difference in migration behaviors, as MCF10a migration will likely be minimal in both 2D and 3D models.

# Chapter 2: Methodology

## Cell Cultures

All cell lines were grown separately in 250 ml culture flasks and maintained in an incubator set to 37°C with 5% $CO_2$. All cell culture medium was based on methods advised by the cell vendors[24,27,29]. All cell culture media used Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen) as the media base. The MDA-MB-231 growth medium was supplemented with 10% volume FBS (Sigma F6178), 1% volume Penicillin-Streptomycin (Invitrogen/Gibco 15140-148) and 1 ml Mycozap solution. The MCF-7 growth medium was supplemented with 10% volume FBS, 1% volume Penicillin-Streptomycin, 1 ml Mycozap solution, and 0.01 mg/ml Recombinant Human Insulin (Sigma 91077C-100MG). The MCF-10a growth medium was supplemented with 5% volume Horse Serum, 1% volume Penicillin-Streptomycin, 20 ng/ml Epidermal Growth Factor (EGF) (Sigma E9644), 0.5 mg/ml Hydrocortisone (Sigma H0888), 50 $\mu$g/ml Cholera Toxin (Sigma C8052), 0.01 mg/ml insulin, and 1 ml MycoZap. Composition of each medium was not taken into consideration for during experiment analyses.

Cell medium was changed every two days, and cells were passaged once cell confluency exceeded 70%. The time intervals between passaging varied between cell lines based on their growth rates.

For passaging, cells were washed with 5 ml phosphate buffered saline (PBS) without calcium and magnesium [Sigma P3813], and then exposed to 3 ml Trypsin-EDTA [Sigma T4049]. Exposure time of the Trypsin-EDTA solution varied among cell lines because of differences in the levels of adherence. Once the cells were no longer adherent to the cell culture flask, 8 ml of medium was added to the cell culture well to dilute the Trypsin-EDTA solution, and the contents of the cell culture flask were transferred a conical tube and centrifuged for 3 minutes at 3500 RPM. The liquid waste was removed, and the cell pellet was resuspended within the conical tube using cell culture medium. 10% of the cells in suspension were deposited into a new 250 ml cell culture flask along with 15 ml of cell culture medium.

## Model Preparation

For model preparation, the work required was divided into three distinct parts: cell staining, hydrogel preparation, and hydrogel well preparation. Several of the tasks were done in tandem with others, and a general schedule for the model preparation process can be seen in Table 1.

| Task | Time to start | Time required | Time at completion |
|---|---|---|---|
| Thaw HA | 0:00 | 20 min | 0:20 |
| Dissolve HA | 0:20 | 20 min | 0:40 |
| Count cells | 0:10 | 30 min | 0:40 |
| Thaw Extralink | 0:35 | 5 min | 0:40 |
| Stain cells HA | 0:40 | 60 min | 1:40 |
| Prepare base gels | 0:40 | 20 min | 1:00 |
| Base gel solidification | 1:00 | 40 min | 1:40 |
| Prepare body gels | 1:40 | 30 min | 2:10 |
| Body gel solidification | 2:10 | 50 min | 3:00 |
| Add media | 3:00 | 5 min | 3:05 |

Table 1: General timetable for each conducted experiment

Cell staining

Cell preparation for experimentation occurred as part of the routine cell passaging procedure. Once the cell pellet was isolated from the liquid waste, the cells were suspended in a conical tube with 1 ml of DMEM solution. 10 µl of cell solution was isolated and diluted by a factor of 10 with 90 µl of DMEM. 10 µl of the diluted cell solution was then used for cell counting. Based on the calculated cell concentration in the 1 ml of cell-suspended DMEM, the necessary volume of cell suspended DMEM was isolated and diluted in DMEM to obtain a 1 ml

solution of 500,000 cells/ml in a microcentrifugal tube. To the 500,000 cells/ml solution, 0.5-1 μl of CellTracker Green CMFDA Dye [ThermoFisher C7025] was added, based on the freshness of the dye. The 500,000 cells/ml solution while in the microcentrifugal tube was then placed in a 37°C and 5% $CO_2$ incubator to allow the dye to adhere to the cells.

Hydrogel preparation

Hydrogel solution was one of the two primary variables within this study. Typically, 20% of the hydrogel solution was composed of the stained 500,000 cells/ml solution, 20% collagen solution (unless the hydrogel were to consist of only HA), and variable amounts of DMEM and HA solution. Solid HA was suspended in 1 ml DMEM and allowed to dissolve before addition to hydrogel solutions. Extralink was added along with the HA solution to allow the HA to crosslink and form polymer chains within the collagen hydrogel. HA and extralink were added in a 4:1 ratio. Tables 2 and 3 display all hydrogel composition parameters used within these experiments.

| Components | Pure Collagen | Coll w/0.1wt% HA | Coll w/0.2wt% HA | 0.1wt% HA | 0.2wt% HA |
|---|---|---|---|---|---|
| Collagen | 20% | 20% | 20% | 0% | 0% |
| DMEM | 80% | 67.5% | 55% | 87.5% | 75% |
| HA | 0% | 10% | 20% | 10% | 20% |
| Extralink | 0% | 2.5% | 5% | 2.5% | 5% |

Table 2: Hydrogel components by percent makeup for the hydrogel base layer

| Components | Pure Collagen | Coll w/0.1wt% HA | Coll w/0.2wt% HA | 0.1wt% HA | 0.2wt% HA |
|---|---|---|---|---|---|
| Collagen | 20% | 20% | 20% | 0% | 0% |
| DMEM | 60% | 47.5% | 35% | 67.5% | 55% |
| Cell Solt. | 20% | 20% | 20% | 20% | 20% |
| HA | 0% | 10% | 20% | 10% | 20% |
| Extralink | 0% | 2.5% | 5% | 2.5% | 5% |

Table 3: Hydrogel components by percent makeup for the 3D encapsulation layer

Hydrogel well preparation

Hydrogels were prepared in 48 well cell culture plates. For both cell encapsulation and cell surface experiments, a hydrogel base layer of 150 μl was formed. The hydrogel base layer was incubated at 37°C for roughly one hour, depending on hydrogel composition, to allow hydrogel components to crosslink and gelate. Once the hydrogel was formed, for cell surface experiments, 1 ml of growth medium and 12,500 cells (25 μl of a 500,000 cell/ml solution) were added to the well. For encapsulation experiments, 100 μl of hydrogel solution containing 100,000 cells/ml was added to the well. Cell-encapsulated hydrogels were allowed to crosslink and gelate for approximately 50 minutes at 37°C. After gelation, 1 ml of growth medium was added to each well. For every experimental run, six conditions were analyzed.

Microscopy

Cells in hydrogels were kept overnight in an incubator set to 37°C and 5% $CO_2$. The wells were then fixed within a microscopy device for imaging. Images of cells were taken at 20x and were used for morphology studies. Time-lapse recordings were taken at 10x magnification and images were taken every 20 minutes for 8 hours.

Analysis methods

All cell images were analyzed using the software NIH ImageJ (NIH). Morphology was analyzed via aspect ratio analysis. Migration studies were analyzed using the third party extension MTrackJ. Pixels travelled by individual cells over the time lapse was converted to μm/hr. Statistical analysis of all data was performed using the software JMP, and non-parametric Wilcoxon multiple comparison tests were used to identify data significance.

# Chapter 3: Results and Discussion

One of the primary goals of this investigation was to evaluate the validity of the collagen/HA hydrogel composite model for the study of breast cancer cells. Here, the results of the migration and morphology analyses will be presented as well as a discussion on the meaning behind each set of results.

## Hydrogel composition effects on migration

From the conducted investigation, an overall difference in migration speeds across all experimental cell lines was observed (Figures 7-8). MDA-MB-231 cells across all conditions moved at speeds of 50 to 90 microns/hour, MCF7 cells moved at 30-60 microns/hour, and MCF10a cells showed no apparent signs of movement. Upon further analysis of the MDA-MB-231 migration data, few significant differences in average migration were found across hydrogel composition trials containing collagen (Figure 7). A significant change in cell behavior was only detected for the 2D 0.1wt% HA-Collagen hydrogel condition. Within hydrogels consisting of HA only and no collagen, migration values began to diverge. For the two conditions tested with absolute HA hydrogels (no

collagen), the cells deposited on the hydrogel surface moved much faster than cells encapsulated within the same hydrogel. Although the two encapsulated conditions were found to be statistically similar, cells deposited on surfaces of hydrogels composed of 0.1wt% HA were found to move significantly faster than those on hydrogels of 0.2wt% HA.

MCF-7 cells exhibited a different response to the addition of HA within the hydrogel experiments that that of MDA-MB-231 cells (Figure 8). MCF-7 cells exhibited reduced migration speed in response to increased HA. In experiments with hydrogels consisting solely of HA, MCF-7 displayed a large increase in migration speeds for three of the four conditions (2D and 3D 0.1wt% HA and 2D 0.2wt% HA) compared to cells cultured in collagen containing hydrogels.

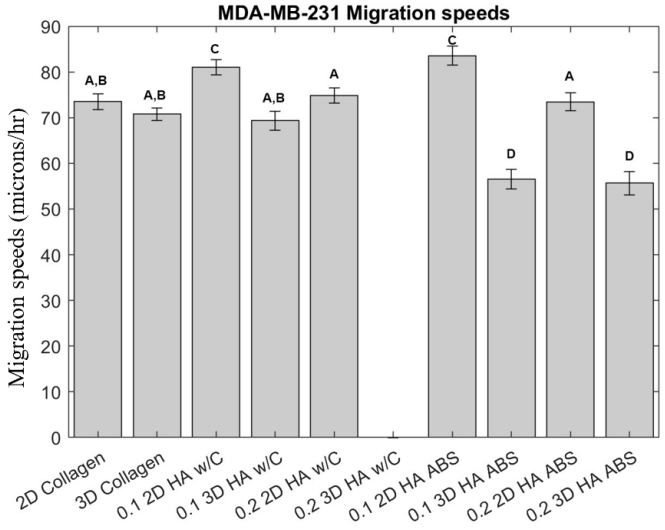


Figure 7: Migration data from MDA-MB-231/hydrogel experiments. Letters indicate statistical similarity between experimental groups (N>90 per data point, p=1.00). Insufficient data for data set 0.2 3D HA ABS due to lab shutdown.

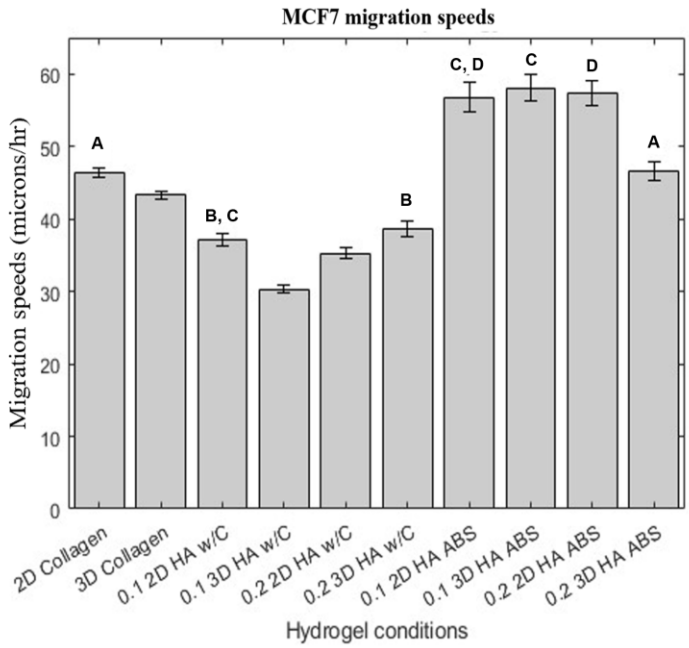


Figure 8: Migration data from MCF-7/hydrogel experiments. Letters indicate statistical similarity between experimental groups (N>130 per data point, p=1.00)

Discussion on migration

Overall migration differences

The overall migration rates for each cell line within this study match expected rates based on recorded characterization of the cell lines[24,28,39]. MDA-MB-231 was the fastest moving cell line across all conditions within collagen or collagen/HA composite hydrogels. MDA-MB-231 is one of the most aggressive, invasive, and well researched breast cancer cell lines studied today[24]. MCF7, which also has been greatly studied over the past 40 years, is less aggressive and is primarily used in primary

tumor growth models[39]. In previous studies, MCF7 has been shown to increase in invasiveness and metastatic tendencies with the addition of estrogen[39]. Since estrogen was not applied in this study, MCF7 was thus expected to behave in a less aggressive manner. Therefore, the experimental results showing MCF7 displaying lower migration rates to that of MDA-MB-231 is consistent with its behavior in other studies as a less invasive cell line.

MCF10a showed little to no signs of movement to such a degree that viable, quantitative migration data could not be collected from MCF10a experiments. This behavior matches the previously recorded behaviors of MCF10a under similar conditions[28]. Images of MCF10a cells, along with the other cell lines, would normally be included to show qualitative differences between lines; however, because of the stay-at-home order during the 2020 COVID-19 quarantine, the pictures of these cells were inaccessible from the laboratory.

### MDA-MB-231 migrational response

These findings from this study suggest that MDA-MB-231 is not hindered by the introduction of HA to a collagen-based hydrogel. These results are in line with the previous understanding of the interaction of CD44 and HA. MDA-MB-231 is a poorly differentiated triple negative breast cancer cell line with a $CD44^{+}/CD24^{-}$ low phenotype[24]. The CD44 protein binds to HA and has roles in cell detachment, stimulating

migration, and promoting invasion[23]. Based on this information, because of CD44 receptor activity in MDA-MB-231 cells, the presence of HA should stimulate cell migration. The significant increase in cell migration for the 2D 0.1wt%HA group in collagen supports this notion, as the presence of HA induced faster cell movement. For the, 3D 0.1wt%HA in collagen, the lack of significant change likely results from the increased stiffness of the hydrogel because of the increased concentration of polymer chains. This increased stiffness could result in an increased amount of resistance within the cell migration path, slowing the cells. For the 2D 0.2wt% HA in collagen, the lack of significant change could be due to the increased amount of matrix degradation required due to the doubled amount of HA within the hydrogels[42].

MCF7 migrational response

Within the collagen and collagen/HA composite hydrogel conditions, MCF7 migration declined significantly with the introduction of HA to collagen-based hydrogel solutions. A previous study showed that MCF7 has low expression of CD44, the cell receptor responsible for cancer cell interaction with HA[40]. A decline in mobility for a CD44-lacking cell line in response to HA exposure compared to a rise in mobility for a CD44-abundant cell supports the theory that CD44 expression positively impacts cell mobility in an environment containing HA.

For hydrogels consisting of only HA and no collagen, the movement of the MCF7 cells was faster and significantly similar across three of the conditions. Because of the overall inability of the MCF7 cells to adhere to HA polymers, this increased rate of migration is likely a result of the MCF7 cells floating within the hydrogel because of a lack of adhesion to the HA infrastructure and because of the movement of the microscope tray during the time-lapse imaging. Without the ability to adhere to their surroundings, cells likely moved involuntarily during the migration study because of the fluid motion in response to the tray movement. This inference is supported by a few recordings that show cells floating towards the meniscus of the hydrogel; however, these recordings were unobtainable for review because of the stay-at-home order during the COVID-19 quarantine.

### Validity of the hydrogel models

The evidence from both MDA-MB-231 and MCF7 experiments support the claim that collagen and collagen/HA composite hydrogel models can reliably be used to investigate cellular migration and invasion responses to the presence of HA. The experimental behavior of MDA-MB-231 and MCF7 cell lines matched the hypothesized behavior of the two cell lines; MDA-MB-231 is characterized a highly aggressive cell line[24] while MCF7 is not[25]. , and the data showed that increased expression of CD44 correlated with increased ability of cells to navigate

polymer matrices containing HA. These models should be viable for further study of cells expressing CD44.

Although HA hydrogel conditions did prove to be beneficial to this study, the protocol for the synthesis of the HA hydrogels will likely need to be reviewed. In this investigation, the HA concentration as well as the time made available for polymer chain crosslinking within the hydrogel, likely were not suitable for forming hydrogels with adequate structural integrity. For this reason, future experiments increasing the HA concentration could prove to be fruitful.

### Validity of the MCF10a cell line

The study of MCF10a within this investigation was explored to compare two breast cancer cell lines, MDA-MB-231 and MCF7, to a normal mammary cell. Although MCF10a did behave in an expected fashion within this study, a previous study has shown that MCF10a cells in 3D culture exhibit unique cellular expressions that do not match that of normal mammary cells *in vivo* [41]. For this reason, MCF10a was useful as a control group, but further use of MCF10a may not be as viable as initially expected.

Hydrogel composition effects on morphology

Aspect ratio was the determinant used to classify cells as either mesenchymal or amoeboid in morphology. Neither MDA-MB-231 nor MCF-7 cells showed a significant pattern in morphology (Figure 9). There was no indicative change in morphology patterns for MDA-MB-231 cells based on the addition of HA to a collagen hydrogel base. There was, however, a decrease in aspect ratio when MDA-MB-231 was exposed to HA-only hydrogel environments. For MCF-7, the aspect ratio declined significantly when the cells were exposed to environments where HA made up a large proportion of the hydrogel matrices (Figure 10).

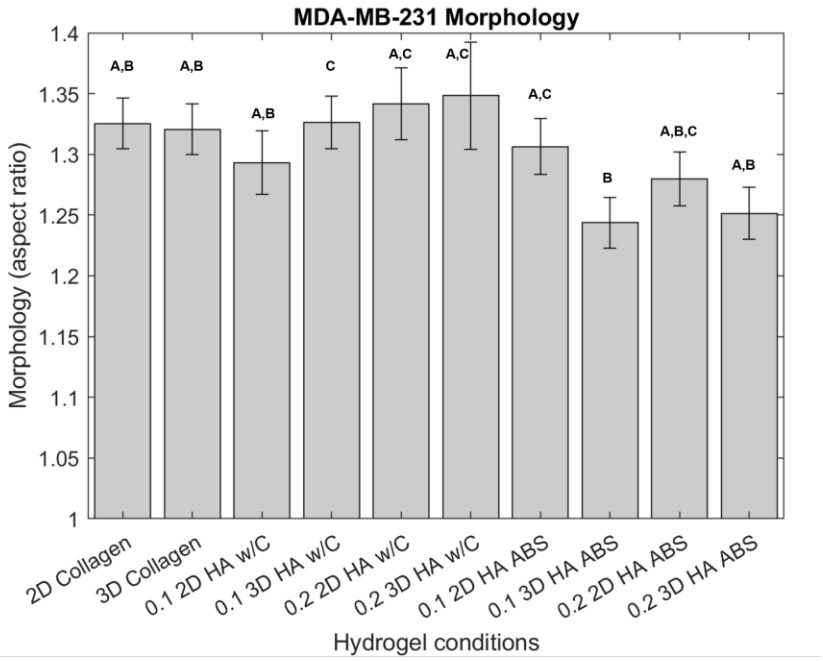


Figure 9: Average aspect ratio values of MDA-MB-231 hydrogel experiments. Letters indicate statistical similarity between experimental groups (N>80 per data point, p=0.7911).

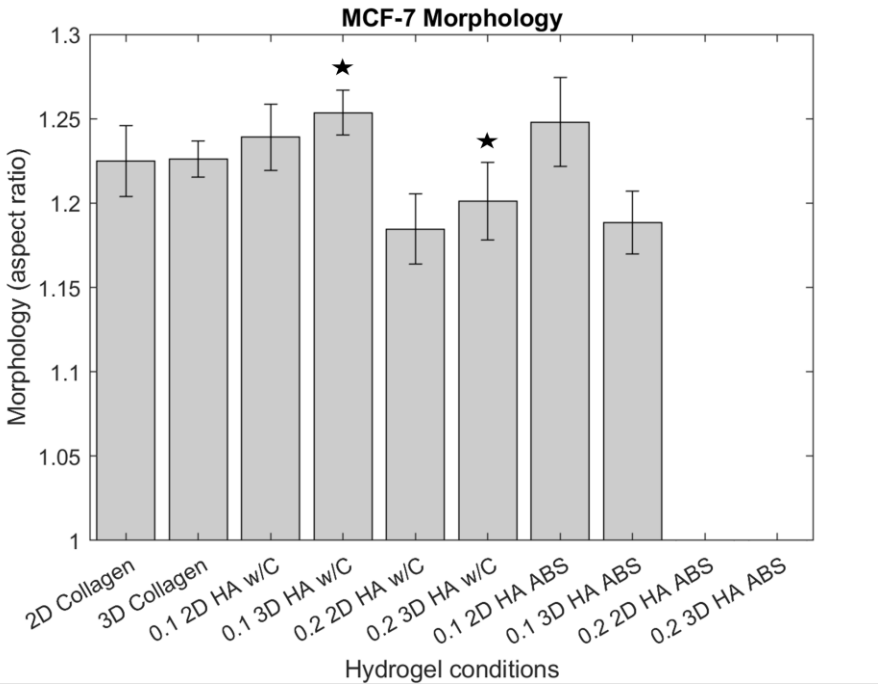


Figure 10: Average aspect ratio values of MCF7 hydrogel experiments. Stars indicate statistical difference between experimental groups (N>100 per data point, p=0.6436). Insufficient data for data sets 0.2 2D HA ABS and 0.2 3D HA ABS due to lab shutdown.

## Discussion on morphology

### Overall morphology response

MDA-MB-231 and MCF-7 cell lines exhibited different ranges of cell morphology. MDA-MB-231 in almost all morphology analyses displayed an average aspect ratio in collagen and collagen/HA composite hydrogels of $> 1.3$. Comparatively, MCF7 did not display an average aspect ratio of $> 1.26$ over any condition. This difference can likely be explained by the inherent differences of the cells: MDA-MB-231 cells are more fibrillar in appearance and were expected to exhibit a more elongated shape with a high AR[24], whereas MCF7 cells are more rounded and thus exhibit morphologies with a lower AR[27].

### MDA-MB-231 morphology response

The results from the MDA-MB-23 morphology analysis reveal that overall morphological behavior of the cell line is independent of the concentration of HA in hydrogel conditions, including collagen. The only significant change in cell morphology occurred in the 3D 0.1wt%HA and 3D 0.2wt%HA hydrogels. This is likely because of a lack of polymer adherence available in comparison to the hydrogels containing collagen; within the pure HA conditions, there was at least a 50% drop in polymer chains within the hydrogel compared to the pure collagen conditions

MCF7 morphology response

For MCF7, a significant decrease in aspect ratio was seen in both the 2D and 3D 0.2wt%HA/collagen composite hydrogel conditions. This can likely be attributed to reduced adhesion locations because of the included HA polymers; MCF7 struggles to bind to HA because of low expression of CD44[40].

## Conclusions

In this study, I examined the variation in cell migration and morphology of the breast cell lines MCF10a, MDA-MB-231, and MCF-7 when encapsulated within hydrogels or deposited on top of a hydrogel base. The hydrogels used in experiments were made from a range of compositions of collagen and/or HA. HA and collagen were chosen as the hydrogel components in order to mimic a breast extracellular environment (i.e., which is composed primarily of collagen), and a brain extracellular environment (i.e., which is composed primarily of HA). The purpose of performing experiments with these hydrogels was to investigate the viability of the hydrogel model, while also furthering understanding of cancer cell migration behaviors. The results of this study showed that highly invasive MDA-MB-231 cells were not hindered by the addition of HA, whereas less invasive MCF-7 cells slowed migration rates in response to the introduction of HA to collagen hydrogels. As a control, MCF10a was also investigated for its morphology and migration patterns in these hydrogels; however, these cells showed no signs of movement for any condition. Based on the results of this study and the literature reported characteristics of each cell

line, these hydrogel models were shown to be promising for studying cell migration behaviors. These hydrogel models can be expanded to investigate cells in environments mimicking other body sites, not just the breast and the brain, through the use of collagen and HA. With an increase in complexity in hydrogels, more intricate models could be considered, suitable for studying cell invasion through a cell barrier or a thick hydrogel barrier.

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